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A generally applicable synthesis of amino acid *p*-nitroanilides as synthons

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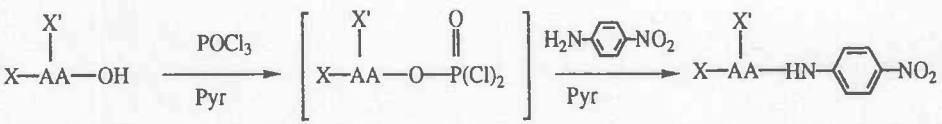
Introduction

Amino acid *p*-nitroanilides are versatile chromogenic substrates for proteolytic enzymes [1]. Their synthesis is problematic because of the low nucleophilicity of *p*-nitroaniline, since the current coupling methods of peptide synthesis appear inadequate. In an earlier paper we mentioned the applicability of phosphorus oxychloride as condensing agent in the synthesis of protected arginine *p*-nitroanilides [2]. We now describe here the use of this reagent in the synthesis of orthogonally protected *p*-nitroanilides and their transformation into synthons for chromogenic substrates.

Results and Discussion

We found that phosphorus oxychloride [3] is an excellent condensing agent for amines and alcohols (J. Broos, personal communication) of low nucleophilicity (Scheme 1). Virtually all Boc- and Z-protected amino acid *p*-nitroanilides were obtained in high yield (70–90%) in an optically pure form, some being given in Table 1. We also found Boc-Ala-pNA to be excellently stable in 50% piperidine in DMF, which prompted us to synthesize Fmoc-amino acid *p*-nitroanilides, which have not been mentioned in the literature until now (Table 1).The use of Fmoc as α-amino protection allows side chain protections which can be cleaved off under mildly acidic conditions at the end of the synthesis. As an example, Scheme 2 depicts the synthesis of the chromogenic substrate (S2238) used in the determination of thrombin.

Bz-Ile-Glu-Gly-Arg-pNA.HCl (S2222), Bz-Ile-Glu(N-piperidyl)-Gly-Arg-pNA.HCl (S2337), from Boc-Arg-pNA.HCl and H-D-Val-Leu-Lys-pNA.2HCl



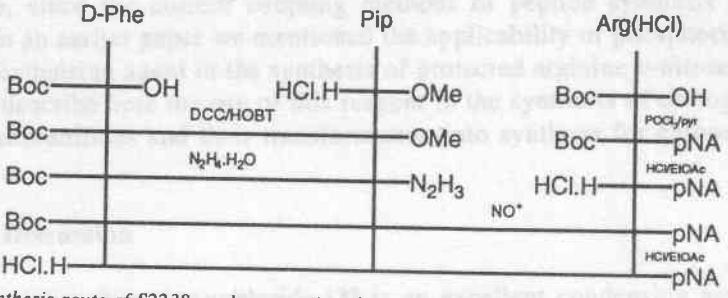
Scheme 1. Equimolar amounts of phosphorus oxychloride and carboxylic acid are required in this procedure. X symbolizes an α-amino protective group, X' a side-chain protection and AA stands for an amino acid.

Table 1 *Some protected amino acid p-nitroanilides sythesized with phosphorus oxychloride as condensing agent*

	Y (%)	m.p. (°C)	α_D		Y (%)	m.p. (°C)	α_D
Boc-Glu(OBzl)-pNA	84	foam	−7.9[a]	Fmoc-Glu(OtBu)-pNA	83	98	−26.8[b]
Boc-Lys(Z)-pNA	84	foam	−8.1[a]	Fmoc-Ser(tBu)-pNA	79	foam	−36.2[b]
Boc-Arg(HCl)-pNA	91	187	−12.8[a]	Fmoc-Tyr-(tBu)-pNA	79	94 (dec.)	+24.5[b]
Z-Arg(HCl)-pNA	96	174	−10.7[a]	Fmoc-Arg(HCl)-pNA	88	96 (dec.)	−45.5[b]
Msc-Arg(HCl)-pNA	65	amorph	−12.4[a]	Fmoc-Lys(Boc)-pNA	81	116–117	−23.3[b]
Fmoc-Gly-pNA	89	212–213		Fmoc-Cys(Trt)-pNA	90	foam	−18.8[b]
Fmoc-Phe-pNA	88	197–199	+13.3[b]	Fmoc-His(Trt)-pNA	95	foam	−6.1[b]
Fmoc-Val-pNA	70	189–191	−27.2[b]	Fmoc-Asn(Trt)-pNA	71	214–215	−25.3[b]
Fmoc-Met-pNA	79	183	−39.1[b]				

[a] c = 1, MeOH.
[b] c = 1, DMF.

(S2251), from Boc-Lys(Z)-pNA or Fmoc-Lys(Boc)-pNA, were synthesized in high yield as substrates for factor X_a and plasmin, respectively, and exhibited the known kinetic data. Our conclusion, that phosphorus oxychloride is a powerful condensing agent in the synthesis of *p*-nitroanilides, is confirmed by these results.



Scheme 2. Synthesis-route of S2238, a chromogenic substrate for thrombin.

References

1. Hemker, H.C. (Ed.) Handbook of Synthetic Substrates for the Coagulation and Fibrinolytic System, Martinus Nijhoff Publishers, Boston, MA, 1983.
2. Rijkers, D.T.S., Hemker, H.C., Nefkens, G.H.L. and Tesser, G.I., Rec. Trav. Chim. Pays-Bas, 10(1991) 347.
3. Used for the first time as coupling reagent in peptide synthesis by Wieland et al., see: Liebigs Ann. Chem., 599(1956)70. The synthesis of an amino acid *p*-nitroanilide with phosphorus oxychloride was for the first time carried out by one of us (G.I.T.) and recommended by Planta, R.J. and Gruber, M., Biochim. Biophys. Acta.